Maternal glucocorticoid metabolism across pregnancy: a potential mechanism underlying fetal glucocorticoid exposure

Maternal glucocorticoid metabolism across pregnancy: a potential mechanism underlying fetal glucocorticoid exposure

David Q. Stoye[1], Ruth Andrew[2], William A. Grobman[3,4], Emma K. Adam[5], Pathik D. Wadhwa[6], Claudia Buss[6,7], Sonja Entringer[6,7], Gregory E. Miller[8], James P. Boardman[1], Jonathan R. Seckl[2], Lauren S. Keenan-Devlin[9], Ann E.B. Borders[4,9], Rebecca M. Reynolds[1,2]

Affiliations: [1]MRC Centre of Reproductive Health, University of Edinburgh, Edinburgh, UK; [2]Centre for Cardiovascular Sciences, University of Edinburgh, Edinburgh, UK; [3]Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; [4]Center for Healthcare Studies, Institute for Public Health and Medicine, Northwestern University, Chicago, IL, USA; [5]School of Education and Social Policy, Institute for Policy Research, Northwestern University, Evanston, IL, USA; [6]Development, Health and Disease Research Program, University of California, Irvine, CA, USA; [7]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health (BIH), Institute of Medical Psychology, Berlin, Germany; [8]Department of Psychology, Institute for Policy Research, Northwestern University, Evanston, IL, USA; [9]Department of Obstetrics and Gynecology, Division of Maternal Fetal Medicine, NorthShore University Health System, University of Chicago Pritzker School of Medicine, Chicago, IL, USA

Short title: Glucocorticoid metabolism across pregnancy

Key words: cortisol, glucocorticoid, metabolism, HPA, pregnancy, birthweight

Corresponding Author: Professor Rebecca M. Reynolds. Centre for Cardiovascular Science, Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh, EH16 4TJ. Telephone: + 44 (0) 131 242 6762. Email: r.reynolds@ed.ac.uk. Reprint requests should be made to Professor Rebecca M. Reynolds.

Funding sources: This project was supported by The National Children's Study, Vanguard Study, Task Order 5 (HHSN275201200007I-HHSN27500005), and Auxiliary Research Scholar and Research Career Development Awards from NorthShore University Health System to Ann E.B. Borders. We acknowledge the support of the British Heart Foundation. David Q. Stoye is supported by a fellowship from Theirworld. This work was undertaken in the MRC Centre for Reproductive Health at the University of Edinburgh, which is funded by MRC Centre Grant (MRC G1002033). We acknowledge the support of the Wellcome Trust (202794/Z/16/Z).

Disclosure summary: The authors have no conflicts of interest relevant to this article to disclose.

Word count (excluding references, tables and figures): 3692

Abstract

Context: Across pregnancy maternal serum cortisol levels rise up to threefold. It is not known whether maternal peripheral cortisol metabolism and clearance change across pregnancy, or influence fetal cortisol exposure and development.

Objectives: The primary study objective was to compare maternal urinary glucocorticoid metabolites, as markers of cortisol metabolism and clearance, between the 2nd and 3rd trimester of pregnancy. Secondary objectives were to test associations of total maternal urinary glucocorticoid excretion, with maternal serum cortisol levels and offspring birthweight z-score.

Design, participants and setting: 151 women with singleton pregnancies, recruited from prenatal clinic at the Pittsburgh site of the Measurement of Maternal Stress (MOMS) study, had 24-hour urine collections during both the 2nd and 3rd trimester.

Results: Between the 2nd and 3rd trimester total urinary glucocorticoid excretion increased (ratio of geometric means (RGM) 1.37, 95% CI 1.22-1.52, $p<0.001$), and there was an increase in calculated 5β-reductase compared to 5α-reductase activity (RGM 3.41, 95% CI 3.04-3.83, $p<0.001$). During the 3rd trimester total urinary glucocorticoid excretion and serum cortisol were negatively correlated ($r=-0.179$, $p=0.029$). Mean total urinary glucocorticoid excretion across both trimesters and offspring birthweight z-score were positively associated ($\beta=0.314$, $p=0.001$).

Conclusions: The estimated activity of maternal enzymes responsible for cortisol metabolism change between the 2nd and 3rd trimester of pregnancy. Additionally, maternal peripheral metabolism and clearance of cortisol may serve as a novel mechanism impacting fetal cortisol exposure and growth.

68 **Précis:** Maternal urine was sampled as part of a pregnancy cohort. Estimated cortisol metabolism
69 changes across pregnancy, and total urinary glucocorticoid excretion is positively associated with fetal
70 growth.

Introduction

Glucocorticoids play a critical role in fetal maturation. While a surge in glucocorticoid exposure towards the end of pregnancy helps prime a fetus for life outside the womb[1], excess or inappropriately timed exposure can adversely programme offspring development[2,3]. There is growing evidence that circulating levels of maternal cortisol influence both fetal cortisol exposure and development. Maternal blood cortisol levels correlate with cortisol levels measured in fetal blood[4] and amniotic fluid[5]. Elevated cortisol levels measured in maternal blood or saliva are associated with offspring growth restriction and adverse neurodevelopment and metabolic health[6-8].

Maternal regulation of glucocorticoids changes profoundly across pregnancy, with circulating cortisol levels rising approximately threefold by delivery[9]. Multiple factors contribute to maternal hypercortisolism including rising cortisol binding globulin (CBG)[10], placental secretion of corticotropin releasing hormone (CRH)[11], and reduced sensitivity of the hypothalamic-pituitary-adrenal (HPA) axis to glucocorticoid mediated central negative feedback[12]. Altered breakdown, clearance and regeneration of cortisol within maternal peripheral tissues could also influence maternal serum levels and fetal glucocorticoid exposure.

Relatively little intact cortisol is excreted from the body passively, with the majority instead being metabolised to compounds considered more inert before urinary excretion[13]. Metabolism of cortisol to 5β-tetrahydrocortisol (THF), and its derivatives α-cortol and β-cortol, and 5α-tetrahydrocortisol (α-THF), are reliant on the activity of A-ring reductases, 5β-reductase, predominantly expressed in the liver, and 5α-reductase, expressed in both liver and fat. 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) acts in the kidney and placenta, converting cortisol to cortisone. In contrast, 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is most highly expressed in the liver, where it regenerates active cortisol from inert cortisone. These processes are outlined in figure 1. Peripheral glucocorticoid metabolism varies as a function of age, gender and obesity and in many disease states[14-16].

The sum of glucocorticoid metabolites measured in a 24-hour sample of urine represents total urinary glucocorticoid excretion. As the majority of glucocorticoids are excreted in urine this measurement has also been used as an estimate of glucocorticoid production by the adrenal gland[17]. Additionally, comparison of the relative levels of metabolites offers insight into the activity of enzymes converting cortisol in peripheral tissues.

To date there has been limited investigation of maternal peripheral glucocorticoid metabolism and clearance in pregnancy. Longitudinal studies of maternal peripheral glucocorticoid metabolism in pregnancy have been limited by small sample size[18], or have relied on metabolites collected in spot urine or blood samples that are subject to diurnal variation[19,20]. There is growing evidence that maternal peripheral glucocorticoid metabolism and clearance are altered in preeclampsia[20-22]. There is also preliminary data supporting a role for peripheral glucocorticoid metabolism influencing fetal development, with a higher plasma cortisone to cortisol ratio (representing more inert compared to active glucocorticoid) measured in mothers with psychiatric morbidity during the 3rd trimester, being associated with higher offspring birthweight[23].

The aims of this study were to assess how maternal urinary glucocorticoid excretion, measured in 24-hour urine, changes between the 2nd and 3rd trimester of pregnancy, and to test the associations of total urinary glucocorticoid excretion with maternal serum cortisol levels and offspring birth weight z-score. We tested the hypothesis that total urinary glucocorticoid excretion, as a marker of maternal adrenal cortisol production, increases across pregnancy, and is negatively associated with offspring birthweight z-score.

Materials and Methods

Participants and clinical protocol

The Measurement of Maternal Stress (MOMS) study was a multisite prospective cohort that recruited women with singleton pregnancies from antenatal clinics in Pittsburgh, PA, Chicago, IL, Schuylkill

County, PA and San Antonio, TX between June 2013 and May 2014. Exclusion criteria were fetal congenital abnormality, chromosomal abnormalities, progesterone use before 14 weeks' gestation, or regular maternal corticosteroid use. All participating women gave written informed consent, and the study protocol was approved by the Institutional Review Board of each site. A description of the cohort has been presented previously[24].

This study reports data from a subset (151 of 200) of mother-baby dyads, recruited from the Pittsburgh site, who had 24-hour urine collected for measurement of total glucocorticoids and metabolites on two occasions during pregnancy, between 12.7 and 22.1 weeks' gestation (2nd trimester), and between 31.9 and 36.4 weeks' gestation (3rd trimester).

Participants also had blood collected for measurement of serum cortisol at study visits during the 2nd and 3rd trimester. Maternal demographic and medical information including body mass index (BMI), age, ethnicity, diabetes mellitus, preeclampsia, gestational hypertension and offspring outcomes including birthweight and birth gestation, were recorded either during study visits, or on review of participants' medical records. Offspring birthweight z-scores were calculated according to International Fetal and Newborn Growth Consortium for the 21st Century (INTERGROWTH-21st) standards[25].

Laboratory methods

Serum

Serum was obtained by centrifuging whole blood at 1000 g at 4 °C for 15 minutes, then aliquoting serum into 2mL cryovials. Cortisol was assessed by radioimmunoassay at the Development, Health and Disease Research Program's laboratory at the University of California, Irvine. 10% of samples where measured in duplicate, and inter-assay and intra-assay CVs were <10%.

Urinary glucocorticoids

Urinary glucocorticoid metabolites were analysed by gas chromatography triple quadrupole mass spectrometry (GC-MS/MS), at the Edinburgh Clinical Research Facility Mass Spectrometry Core as previously described[26]. The inter- and intra-assay CVs were <13%. Analytes included cortisol (F), cortisone (E), α-THF, THF, α-cortol, β-cortol, THE, α-cortolone and β-cortolone. The sum of these measured analytes is referred to as total urinary glucocorticoid excretion.

The following ratios of urinary metabolites were used as parameters to estimate peripheral glucocorticoid metabolism:

i) 11β-HSD2 activity = F / E

ii) 11β-HSD total activity = (THF + α-THF) / THE.

iii) Relative 5β-reductase and 5α-reductase activity = THF / α-THF

iv) 5α-reductase activity = F / α-THF

v) 5β-reductase metabolism of F = F / (THF + α-cortol + β-cortol)

vi) 5β-reductase metabolism of E = E / (THE + α-cortolone + β-cortolone)

Statistical Analysis

All analyses were performed using IBM SPSS Statistics Version 24. Data distributions were assessed for normality visually using histograms. Serum cortisol levels were normally distributed amongst the study population. Levels of all excreted urinary glucocorticoid metabolites were positively skewed, and log base 10 transformed prior to statistical analysis.

Demographic data is presented as mean ± SD. Change of urinary metabolite excretion between the 2nd and 3rd trimester was tested using paired *t* tests, and the degree of change is represented through the ratio of the geometric means (RGM), with 95% confidence intervals. To assess if peripheral metabolism has a maintained trait component across pregnancy, the rank stability, i.e. the similarity of where participants' estimated enzymatic function fell within the study population's distribution, at the 2nd compared to the 3rd trimester, was tested by a linear regression model adjusting for the gestation of urine sampling. The relationship between maternal total urinary glucocorticoid excretion and serum cortisol

levels was tested using Pearson's Coefficient within both the whole study population and in a subgroup of patients with blood sampled before 10 am. Finally, the association of maternal total urinary glucocorticoid excretion and offspring birthweight z-score was tested by linear regression adjusting for confounding factors. These included the gestation at urine sampling and maternal ethnicity, smoking status, age, preeclampsia, gestational hypertension, diabetes mellitus (pre-gestational and gestational), BMI and gravidity. Associations with birthweight z-score were tested for both 2nd and 3rd trimester glucocorticoid excretion, and for mean glucocorticoid excretion across pregnancy. A p-value < 0.05 was considered statistically significant.

Results

Demographics

Table 1 shows the characteristics of study participants. Mothers were aged 30.5 ± 5.0 years, with BMI 27.6 ± 7.1 kg/m^2, and were predominantly white non-smokers. Mean gestational age at birth was 39.4 ± 1.4 weeks, and mean birthweight was 3487 ± 489 grams.

Changing glucocorticoid levels across pregnancy

Figure 2 and table 2 depict urinary glucocorticoid metabolite excretion for collections during the 2nd and 3rd trimester. Across pregnancy total urinary glucocorticoid excretion increased (RGM 1.37, p<0.001). Excretion of all individual metabolites increased except for α-THF which decreased between the 2nd and 3rd trimester (RGM 0.55, p<0.001). Assessing individual metabolic pathways, the ratio of F / E (RGM 0.90, p<0.001) decreased likely representing increased estimated 11β-HSD2 (inactivation of cortisol to cortisone) activity across pregnancy. Total body 11β-HSD activity represented by (THF + α-THF) / THE (RGM 1.27, p<0.001) shifted in favour of excretion of cortisol metabolites relative to cortisone metabolites. The activity of A-ring reductases shifted towards 5β-reductase metabolism compared to 5α-reductase metabolism with increased THF / α-THF ratio (RGM 3.41, p<0.001). Between the 2nd and 3rd trimester serum cortisol also increased (ratio of means 1.63, 95% CI 1.40-1.85, p<0.001).

Individual stability in peripheral glucocorticoid metabolism

Table 3 and figure 3 represent rank-order stability of total urinary glucocorticoid excretion and estimates of peripheral metabolism of glucocorticoids for participants across the 2nd and 3rd trimester. Despite the whole group changes in peripheral glucocorticoid metabolism across pregnancy the relative enzymatic activity of individual participants compared to the whole group was well maintained across both time points, with women with higher estimated activity for peripheral glucocorticoid metabolism during the 2nd trimester tending to have higher estimated enzyme activity measured in the third trimester.

Associations between total urinary glucocorticoid excretion and serum cortisol levels

During the 2nd trimester serum cortisol was not associated with total urinary glucocorticoid excretion ($r=0.076$, $p=0.358$). During the 3rd trimester, total urinary glucocorticoid excretion was negatively associated with serum cortisol within the whole group ($r=-0.179$, $p=0.029$). This association between 3rd trimester serum cortisol and total urinary glucocorticoid excretion was largely driven by the subgroup of participants with 3rd trimester blood samples taken before 10am ($n=66$, $r=-0.354$, $p=0.004$). In contrast, for participants with 3rd trimester blood taken after 10am ($n=83$, $r=-0.096$, $p= 0.390$).

Associations between total urinary glucocorticoid excretion and infant birthweight z-score

In the adjusted models, there were positive associations between total urinary glucocorticoid excretion during the 2nd trimester and offspring birth weight z-score ($\beta=0.198$, r-square change 0.028, $p=0.033$), total urinary glucocorticoid excretion during the 3nd trimester and offspring birth weight z-score ($\beta=0.202$, r-square change 0.032, $p=0.023$), and mean total glucocorticoid excretion across both trimesters with offspring birth weight z-score ($\beta=0.314$, r-square change 0.066, $p=0.001$). In contrast, there was no association between mean serum cortisol levels and offspring birthweight z-score. A visual representation of maternal glucocorticoid excretion across trimesters according to infant birthweight quantile is shown in figure 4.

Associations between glucocorticoid metabolite ratios, with serum cortisol and infant birthweight z-score

Having demonstrated that total urinary glucocorticoid excretion was negatively associated with serum cortisol during the 3rd trimester and positively associated with birthweight z-score, further exploratory analysis was undertaken to investigate whether these effects were being driven by the action of individual metabolic pathways. In this exploratory analysis, higher 3rd trimester serum cortisol was associated with estimates of reduced 5α-reductase activity (F / α-THF; whole group r=0.168, p=0.041; venepuncture <10am subgroup r=0.318, p=0.009), and reduced 5β-reductase activity (F / (THF + α-cortol + β-cortol); whole group r=0.206, p=0.012; venepuncture <10am subgroup r=0.281, p=0.022) and (E / (THE + α-cortolone + β-cortolone); whole group r=0.252, p=0.002; venepuncture <10am subgroup r=0.251, p=0.042). No associations were seen between 3rd trimester serum cortisol and estimated 11β-HSD1 or 11β-HSD2 activity. Additionally, no association were seen between infant birthweight z-score and urine metabolite ratios.

Discussion

In this study of pregnant women with detailed measurements of glucocorticoid metabolism we have demonstrated that glucocorticoid metabolism changes across pregnancy, and that total urinary glucocorticoid excretion is positively associated with offspring birthweight z-score.

Within the cohort total maternal glucocorticoid excretion increased between the 2nd and 3rd trimester. This builds on previous observations of increased urinary free cortisol excretion across pregnancy[9], and likely represents an increase in adrenal cortisol release across pregnancy. There were also differences in the ratios of urinary metabolites between the 2nd and 3rd trimester. This provides evidence that the global actions of enzymes working to metabolise cortisol in peripheral tissues changes across pregnancy. A reduced F/E ratio represents increased 11β-HSD2 activity. An increase in (THF + α-THF) / THE ratio, in the context of estimated increased 11β-HSD2 likely represents an increase in 11β-HSD1

activity across pregnancy. The ratio of A-ring reductase metabolism shifted profoundly towards 5β-reductase meta[27]bolism compared to 5α-reductase metabolism with increased THF / α-THF ratio. A reduction of 5α-reductase cortisol metabolism is in keeping with results from a study where α-THF excretion measured in maternal urine rose across the first year postpartum[28]. The action of 5α-reductase in pregnancy has received attention due to its important role in converting testosterone to dihydrotestosterone, with 5α-reductase genetic mutation or pharmacological inhibition causing *in utero* under-virilization of male offspring[29]. 5α-reductase metabolism of progesterone has also been investigated in the context of parturition, with 5α-reductase type 1 deficient mice failing to undergo cervical ripening at term[30]. However, to our knowledge the physiological importance of 5α-reductase metabolism of cortisol in pregnancy has not previously been considered.

Changes in glucocorticoid metabolism may offer specific advantages to the mother and fetus. In addition to controlling systemic cortisol inactivation and clearance, peripherally located enzymes play an important role in regulating glucocorticoid exposure to specific tissues. This is most commonly discussed in relation to the kidney, where local 11β-HSD2 acts to prevent excessive activation of mineralocorticoid receptors by cortisol[13]. 5α-reductase influences cortisol clearance and action within the liver, and its activity has been shown to be modifiable either by early life stress[31], or by variation in nutritional demands[32,33]. Within pregnancy, marked reduction in 5α-reductase activity during the 3rd trimester may act to enhance cortisol activity in the liver, allowing mobilisation of fuels at a time of increased metabolic requirements.

Alternatively, changing glucocorticoid metabolism across pregnancy may be a bystander influenced by other physiological changes in the mother across pregnancy. Maternal glucocorticoid metabolism could be influenced by a changing inflammatory milieu. For example it has both been demonstrated that tumor necrosis factor alpha (TNF-α) rises across pregnancy[27], and that inhibiting TNFa in patients with inflammatory arthritis increases 5α-reductase activity[34]. Changing biliary physiology may also influence maternal glucocorticoid metabolism, with bile acids holding the potential to inhibit A-ring reductases and 11β-HSDs[35]. Increases in insulin resistance across pregnancy may also influence

293 glucocorticoid metabolism. However, insulin sensitizing therapies and weight loss have both previously
294 been associated with decreases in 5α-reductase activity[36,37], making it unlikely that changes in insulin
295 sensitivity are driving the reductions in 5α-reductase activity seen within the 3rd trimester. There is also
296 likely to be a placental contribution to maternal whole-body glucocorticoid metabolism estimated
297 through urinary glucocorticoids. In an ex vivo placental perfusion model the majority of cortisone
298 converted from cortisol at term gestation was transferred back into the maternal circulation rather than
299 fetal circulation[38].
300
301 During the 2nd trimester there was no association between maternal urinary glucocorticoid excretion
302 and serum cortisol, whilst during the 3rd trimester higher serum cortisol correlated with lower total
303 urinary glucocorticoid excretion. Additionally, in exploratory analysis, higher serum cortisol in the third
304 trimester was associated with lower estimated activity of 5β-reductase and 5α-reductase. Individual
305 differences in peripheral glucocorticoid metabolism and clearance may influence serum cortisol levels
306 in the later stages of pregnancy. In healthy non-pregnant populations differences in peripheral
307 glucocorticoid metabolism are generally not associated with serum cortisol levels, likely due to
308 compensatory glucocorticoid release by the HPA axis in response to changing negative feedback[39,40].
309 However in critically ill patients reduced peripheral metabolism and clearance of cortisol contributes to
310 raised serum cortisol levels[16]. Throughout pregnancy regulation of the maternal HPA axis changes,
311 becoming progressively less sensitive to negative feedback by glucocorticoids[12]. It therefore seems
312 physiologically plausible that by the 3rd trimester individual differences in glucocorticoid metabolism
313 and clearance influence serum cortisol levels.
314
315 An unexpected finding was the modest positive association between total urinary glucocorticoid
316 excretion and offspring birthweight z-score, with maternal total urinary glucocorticoid excretion
317 measured in the 2nd and 3rd trimesters of pregnancy explaining 6.6% of variance in offspring birthweight
318 z-score. Previous studies have typically reported a negative association between synthetic
319 glucocorticoid exposure[2], or maternal cortisol levels measured in saliva[7] or blood[41], with infant
320 birthweight. A negative association has also previously been reported between urinary free cortisol

measured in the morning between 18-20 weeks' gestation and fetal growth[42]. The relationship between total urinary glucocorticoid excretion and infant birthweight z-score has not previously been tested. Increased maternal peripheral metabolism and clearance of glucocorticoids may serve as a mechanism reducing cortisol exposure to the fetus. This theory is strengthened by the negative association found between serum cortisol and total urinary glucocorticoids observed in the third trimester. In the exploratory analyse no associations were found between birthweight z-score and any of the urinary metabolite ratios used to estimate peripheral enzymatic function, and so it cannot be concluded that this relationship is driven through the effects of a single enzyme's function. Alternatively, the relationship between maternal total urinary glucocorticoid excretion and infant birthweight z-score could be mediated by other maternal factors. For example, increased urinary glucocorticoid excretion has previously been associated with insulin resistance[36], and increased maternal insulin resistance during pregnancy may also act to increase offspring birthweight[43].

Despite whole group changes in peripheral metabolism across pregnancy, individuals' rank within the cohort remained relatively stable with those who had higher calculated enzymatic activity during the 2nd trimester also tending to have higher activity during the 3rd trimester. This implies that individual's peripheral metabolism shows a consistent trait across pregnancy, increasing the likelihood that peripheral glucocorticoid metabolism could influence fetal exposure to cortisol, and play a role in fetal development.

Strengths of this study include the use of a modern technique for accurate quantification of urinary glucocorticoid metabolites[26], the large sample size, and longitudinal study design allowing comparison of urinary metabolites across pregnancy. Limitations include the fact that there was variation in the time of day blood samples were collected, that participants did not fast before venepuncture, and the lack of measurement of other serum glucocorticoid metabolites in addition to cortisol.

Conclusions

Between the 2nd and 3rd trimester the ratios of urinary glucocorticoids, acting as markers of peripheral metabolism, changed suggesting a relative decrease in 5α-reductase metabolism and relative increase in 5β-reductase metabolism of cortisol. However inter-individual differences among study participants were relatively well preserved between the two testing periods. The negative association between total urinary glucocorticoids and 3rd trimester serum cortisol, along with the positive association between total urinary glucocorticoids and birthweight z-score, provides preliminary data that peripheral glucocorticoid metabolism may influence fetal glucocorticoid exposure and fetal growth.

Acknowledgements

We are grateful for the support of the MOMS Study Collaboration including research staff and participants. We also appreciate the important contribution of the MOM-led pilot study collaboration. We acknowledge the support of the University of Edinburgh Mass Spectrometry Core.

Data Availability

The dataset generated during the current study is not publicly available but is available from the corresponding author on reasonable request.

References

1. Fowden AL, Li J, Forhead AJ. Glucocorticoids and the preparation for life after birth: are there long-term consequences of the life insurance? *The Proceedings of the Nutrition Society.* 1998;57(1):113-122.
2. Reynolds RM. Glucocorticoid excess and the developmental origins of disease: two decades of testing the hypothesis–2012 Curt Richter Award Winner. *Psychoneuroendocrinology.* 2013;38(1):1-11.
3. Moisiadis VG, Matthews SG. Glucocorticoids and fetal programming part 1: Outcomes. *Nat Rev Endocrinol.* 2014;10(7):391-402.
4. Gitau R, Cameron A, Fisk NM, Glover V. Fetal exposure to maternal cortisol. *Lancet (London, England).* 1998;352(9129):707-708.
5. Glover V, Bergman K, Sarkar P, O'Connor TG. Association between maternal and amniotic fluid cortisol is moderated by maternal anxiety. *Psychoneuroendocrinology.* 2009;34(3):430-435.

6. Zijlmans MA, Riksen-Walraven JM, de Weerth C. Associations between maternal prenatal cortisol concentrations and child outcomes: A systematic review. *Neuroscience and biobehavioral reviews.* 2015;53:1-24.
7. Cherak SJ, Giesbrecht GF, Metcalfe A, Ronksley PE, Malebranche ME. The effect of gestational period on the association between maternal prenatal salivary cortisol and birth weight: A systematic review and meta-analysis. *Psychoneuroendocrinology.* 2018;94:49-62.
8. Stinson LJ, Stroud LR, Buka SL, et al. Prospective evaluation of associations between prenatal cortisol and adulthood coronary heart disease risk: the New England family study. *Psychosom Med.* 2015;77(3):237-245.
9. Jung C, Ho JT, Torpy DJ, et al. A longitudinal study of plasma and urinary cortisol in pregnancy and postpartum. *J Clin Endocrinol Metab.* 2011;96(5):1533-1540.
10. Qureshi AC, Bahri A, Breen LA, et al. The influence of the route of oestrogen administration on serum levels of cortisol-binding globulin and total cortisol. *Clin Endocrinol (Oxf).* 2007;66(5):632-635.
11. Sasaki A, Shinkawa O, Yoshinaga K. Placental corticotropin-releasing hormone may be a stimulator of maternal pituitary adrenocorticotropic hormone secretion in humans. *Journal of Clinical Investigation.* 1989;84(6):1997-2001.
12. Odagiri EMI, Ishiwatari N, Abe Y, et al. Hypercortisolism and the Resistance to Dexamethasone Suppression during Gestation. *Endocrinologia Japonica.* 1988;35(5):685-690.
13. Walker BR, Seckl JR. Cortisol metabolism. *International Textbook of Obesity.* 2001:241-268.
14. Nixon M, Upreti R, Andrew R. 5α-Reduced glucocorticoids: a story of natural selection. *J Endocrinol.* 2012;212(2):111-127.
15. Andrew R, Phillips DI, Walker BR. Obesity and gender influence cortisol secretion and metabolism in man. *J Clin Endocrinol Metab.* 1998;83(5):1806-1809.
16. Boonen E, Vervenne H, Meersseman P, et al. Reduced Cortisol Metabolism during Critical Illness. *New England Journal of Medicine.* 2013;368(16):1477-1488.
17. Remer T, Maser-Gluth C, Wudy SA. Glucocorticoid measurements in health and disease--metabolic implications and the potential of 24-h urine analyses. *Mini reviews in medicinal chemistry.* 2008;8(2):153-170.
18. Stirrat LI, O'Reilly JR, Riley SC, et al. Altered maternal hypothalamic-pituitary-adrenal axis activity in obese pregnancy is associated with macrosomia and prolonged pregnancy. *Pregnancy Hypertens.* 2014;4(3):238.
19. Mistry HD, Eisele N, Escher G, et al. Gestation-specific reference intervals for comprehensive spot urinary steroid hormone metabolite analysis in normal singleton pregnancy and 6 weeks postpartum. *Reprod Biol Endocrinol.* 2015;13:101.
20. Vasku M, Kleine-Eggebrecht N, Rath W, Mohaupt MG, Escher G, Pecks U. Apparent systemic 11ß-dehydroxysteroid dehydrogenase 2 activity is increased in preeclampsia but not in intrauterine growth restriction. *Pregnancy Hypertension.* 2018;11:7-11.
21. Jayasuriya NA, Hughes AE, Sovio U, Cook E, Charnock-Jones DS, Smith GCS. A Lower Maternal Cortisol-to-Cortisone Ratio Precedes Clinical Diagnosis of Preterm and Term Preeclampsia by Many Weeks. *J Clin Endocrinol Metab.* 2019;104(6):2355-2366.
22. Kosicka K, Siemiątkowska A, Szpera-Goździewicz A, Krzyścin M, Bręborowicz GH, Główka FK. Increased cortisol metabolism in women with pregnancy-related hypertension. *Endocrine.* 2018;61(1):125-133.
23. Hellgren C, Edvinsson Å, Olivier JD, et al. Tandem mass spectrometry determined maternal cortisone to cortisol ratio and psychiatric morbidity during pregnancy−interaction with birth weight. *Psychoneuroendocrinology.* 2016;69:142-149.
24. Miller GE, Culhane J, Grobman W, et al. Mothers' childhood hardship forecasts adverse pregnancy outcomes: Role of inflammatory, lifestyle, and psychosocial pathways. *Brain Behav Immun.* 2017;65:11-19.
25. Villar J, Ismail LC, Victora CG, et al. International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project. *The Lancet.* 2014;384(9946):857-868.

26. Homer N, Kothiya S, Rutter A, Walker BR, Andrew R. Gas chromatography tandem mass spectrometry offers advantages for urinary steroids analysis. *Analytical Biochemistry.* 2017;538:34-37.
27. Beckmann I, Visser W, Struijk PC, van Dooren M, Glavimans J, Wallenburg HCS. Circulating bioactive tumor necrosis factor-α, tumor necrosis factor-α receptors, fibronectin, and tumor necrosis factor-α inducible cell adhesion molecule VCAM-1 in uncomplicated pregnancy. *American Journal of Obstetrics and Gynecology.* 1997;177(5):1247-1252.
28. Rogers SL, Hughes BA, Jones CA, et al. Diminished 11beta-hydroxysteroid dehydrogenase type 2 activity is associated with decreased weight and weight gain across the first year of life. *J Clin Endocrinol Metab.* 2014;99(5):E821-831.
29. Imperato-McGinley J, Guerrero L, Gautier T, Peterson RE. Steroid 5alpha-reductase deficiency in man: an inherited form of male pseudohermaphroditism. *Science.* 1974;186(4170):1213-1215.
30. Mahendroo MS, Porter A, Russell DW, Word RA. The parturition defect in steroid 5alpha-reductase type 1 knockout mice is due to impaired cervical ripening. *Molecular endocrinology (Baltimore, Md).* 1999;13(6):981-992.
31. Yehuda R, Bierer LM, Andrew R, Schmeidler J, Seckl JR. Enduring effects of severe developmental adversity, including nutritional deprivation, on cortisol metabolism in aging Holocaust survivors. *Journal of psychiatric research.* 2009;43(9):877-883.
32. Tomlinson JW, Finney J, Gay C, Hughes BA, Hughes SV, Stewart PM. Impaired Glucose Tolerance and Insulin Resistance Are Associated With Increased Adipose 11β-Hydroxysteroid Dehydrogenase Type 1 Expression and Elevated Hepatic 5α-Reductase Activity. *Diabetes.* 2008;57(10):2652-2660.
33. Stimson RH, Johnstone AM, Homer NZM, et al. Dietary Macronutrient Content Alters Cortisol Metabolism Independently of Body Weight Changes in Obese Men. *The Journal of Clinical Endocrinology & Metabolism.* 2007;92(11):4480-4484.
34. Nanus DE, Filer AD, Hughes B, et al. TNFalpha regulates cortisol metabolism in vivo in patients with inflammatory arthritis. *Annals of the rheumatic diseases.* 2015;74(2):464-469.
35. McNeilly AD, Macfarlane DP, O'Flaherty E, et al. Bile acids modulate glucocorticoid metabolism and the hypothalamic-pituitary-adrenal axis in obstructive jaundice. *Journal of hepatology.* 2010;52(5):705-711.
36. Tomlinson JW, Finney J, Hughes BA, Hughes SV, Stewart PM. Reduced glucocorticoid production rate, decreased 5alpha-reductase activity, and adipose tissue insulin sensitization after weight loss. *Diabetes.* 2008;57(6):1536-1543.
37. Glintborg D, Hermann AP, Hagen C, et al. A randomized placebo-controlled study on the effects of pioglitazone on cortisol metabolism in polycystic ovary syndrome. *Fertility and sterility.* 2009;91(3):842-850.
38. Stirrat LI, Sengers BG, Norman JE, et al. Transfer and Metabolism of Cortisol by the Isolated Perfused Human Placenta. *J Clin Endocrinol Metab.* 2018;103(2):640-648.
39. Purnell JQ, Brandon DD, Isabelle LM, Loriaux DL, Samuels MH. Association of 24-hour cortisol production rates, cortisol-binding globulin, and plasma-free cortisol levels with body composition, leptin levels, and aging in adult men and women. *J Clin Endocrinol Metab.* 2004;89(1):281-287.
40. White PC, Mune T, Agarwal AK. 11β-Hydroxysteroid Dehydrogenase and the Syndrome of Apparent Mineralocorticoid Excess*. *Endocrine Reviews.* 1997;18(1):135-156.
41. Goedhart G, Vrijkotte TG, Roseboom TJ, van der Wal MF, Cuijpers P, Bonsel GJ. Maternal cortisol and offspring birthweight: results from a large prospective cohort study. *Psychoneuroendocrinology.* 2010;35(5):644-652.
42. Diego MA, Field T, Hernandez-Reif M, Schanberg S, Kuhn C, Gonzalez-Quintero VH. Prenatal depression restricts fetal growth. *Early Human Development.* 2009;85(1):65-70.
43. Yamashita H, Yasuhi I, Fukuda M, et al. The association between maternal insulin resistance in mid-pregnancy and neonatal birthweight in uncomplicated pregnancies. *Endocr J.* 2014;61(10):1019-1024.

Figure legends

Figure 1. Peripheral cortisol metabolism enzymes and metabolites

Figure 2. Geometric mean and 95% confidence intervals of glucocorticoid metabolites from 24-hour urine collections during the 2nd and 3rd trimester. * p<0.01, ** p<0.001

Figure 3. Rank correlation across the 2nd and 3rd trimesters of participant total urinary glucocorticoid excretion or estimated enzymatic function, ** p<001

Figure 4. Geometric means and 95% confidence intervals of mothers' mean total urinary glucocorticoid excretion across trimesters according to offspring birthweight z-score quintile

Table Legends

Table 1. Maternal, infant and sampling demographics

Table 2. Changes in urinary metabolites excretion and ratios across pregnancy

Table 3. Rank Correlation across the 2nd and 3rd trimesters of participant total urinary glucocorticoid excretion or estimated enzymatic function

510 **Table 1. Maternal, infant and sampling demographics**

Maternal demographics	Number (%), Mean ± SD
Maternal Age (years)	30.5 ± 5.0
Maternal BMI (kg/m^2)	27.6 ± 7.1
Gravidity	
-1	50 (33.1%)
-2	41 (27.2%)
-≥3	60 (39.7%)
Ethnicity	
-Hispanic White	1 (0.7%)
-White	118 (78.1%)
-Black	27 (17.9%)
-Other	5 (3.3%)
Current Smoker	
-Yes	10 (6.6%)
-No	141 (93.4%)
Preeclampsia	
-Yes	4 (2.8%)
-No	139 (97.2%)
Hypertension	
-Yes	15 (10.5%)
-No	128 (89.5%)
Diabetes	
-Yes	9 (6.3%)
-No	134 (93.7%)
Infant Demographics	
Infant sex	
-Female	61 (42.7%)
-Male	82 (57.3%)
Birthweight (grams)	3487 ± 489
Birth gestation (weeks)	39.4 ± 1.4
Birthweight Z-Score	0.56 ± 0.99
Sampling Demographics	
2nd trimester urine sample gestation (weeks)	17.3 ± 2.4
3rd trimester urine sample gestation (weeks)	33.9 ±1.2
2nd trimester blood sample gestation (weeks)	16.7 ± 2.4
3rd trimester blood sample gestation (weeks)	33.3 ± 1.1
2nd trimester blood sample time (hours after midnight)	11.0 ± 2.2
3rd trimester blood sample time (hours after midnight)	10.6 ±2.5

511

512 Of the 151 participants included in the study the following data was missing: maternal BMI n = 2,

513 infant demographics and maternal health during pregnancy n = 8, 2nd trimester serum cortisol n = 1,

514 3rd trimester serum cortisol n = 2.

515

516 **Table 2. Changes in urinary metabolites excretion and ratios across pregnancy**

	2nd Trimester: Median (lower quartile-upper quartile)	3rd Trimester: Median (lower quartile-upper quartile)	Change across gestations: RGM (95% CI)
Urinary metabolites (Mg / 24 hours)			
THF	1043 (691-1397)	1768 (1066-3269)	1.88 (1.65 to 2.15)[2]
α-THF	494 (331-781)	291 (177-436)	0.55 (0.50 to 0.61)[2]
THE	2500 (1588-3579)	2799 (1805-4222)	1.13 (1.04 to 1.23)[1]
α-cortol	586 (368-917)	641 (455-1140)	1.19 (1.05 to 1.34)[1]
β-cortol	545 (259-947)	849 (540-1410)	1.65 (1.45 to 1.88)[2]
α-cortolone	2420 (1589-4473)	3685 (2371-6241)	1.46 (1.25 to 1.71)[2]
β-cortolone	632 (424-979)	796 (574-1189)	1.29 (1.13 to 1.47)[2]
F	231 (160-315)	272 (215-361)	1.23 (1.13 to 1.35)[2]
E	228 (171-292)	316 (227-410)	1.36 (1.26 to 1.48)[2]
Total urinary glucocorticoids	9691 (6157-12805)	13523 (8955-18269)	1.37 (1.22 to 1.52)[2]
Ratios of metabolites			
11β-HSD2 activity = F / E	0.99 (0.78-1.28)	0.88 (0.73-1.16)	0.90 (0.86 to 0.95)[2]
11β-HSD total activity = (THF + α-THF) / THE	0.61 (0.52-0.85)	0.76 (0.48-1.23)	1.27 (1.14 to 1.42)[2]
Relative 5β -reductase and 5α -reductase activity = THF / α-THF	1.78 (1.33-2.83)	7.19 (3.64-11.74)	3.41 (3.04 to 3.83)[2]
5α -reductase activity = F / α-THF	0.45 (0.27-0.60)	0.98 (0.61-1.51)	2.24 (2.00 to 2.50)[2]
5β-reductase metabolism of F = F / (THF + α-cortol + β-cortol)	0.10 (0.07-0.14)	0.07 (0.05-0.11)	0.72 (0.65 to 0.81)[2]
5β-reductase metabolism of E = E / (THE + +α-cortolone+ β-cortolone)	0.04 (0.02-0.06)	0.04 (0.03-0.06)	1.05 (0.96 to 1.15)

517

518 Paired T-Test (2-tailed) of log transformed urine values. [1]$p < 0.01$, [2]$p<0.001$

519

520

521

522

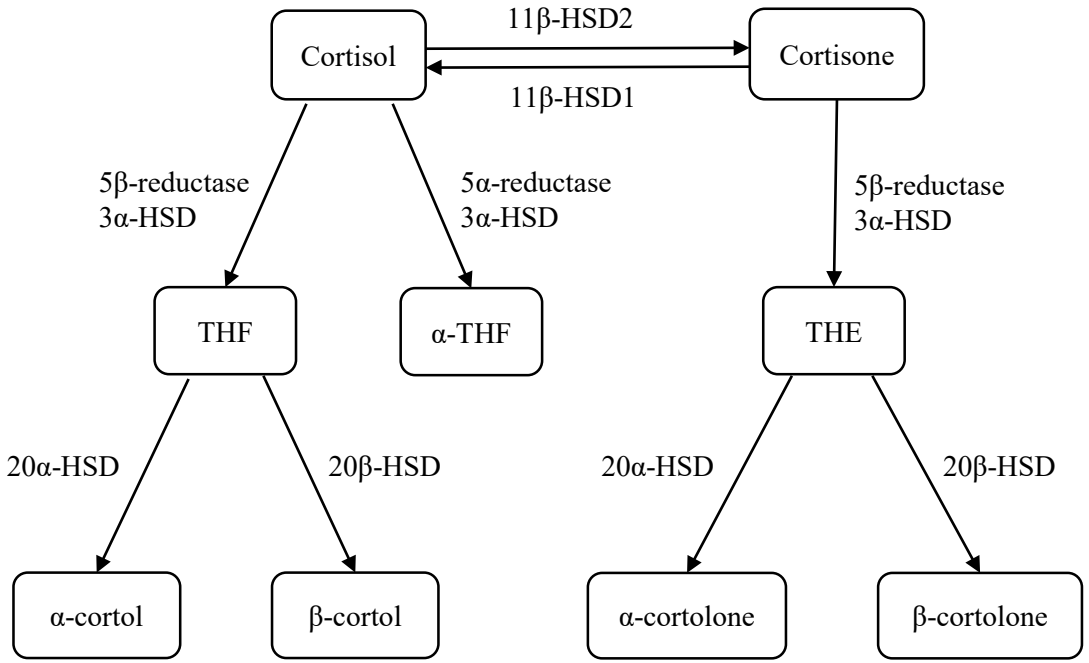
523 **Table 3. Rank Correlation across the 2nd and 3rd trimesters of participant total urinary**

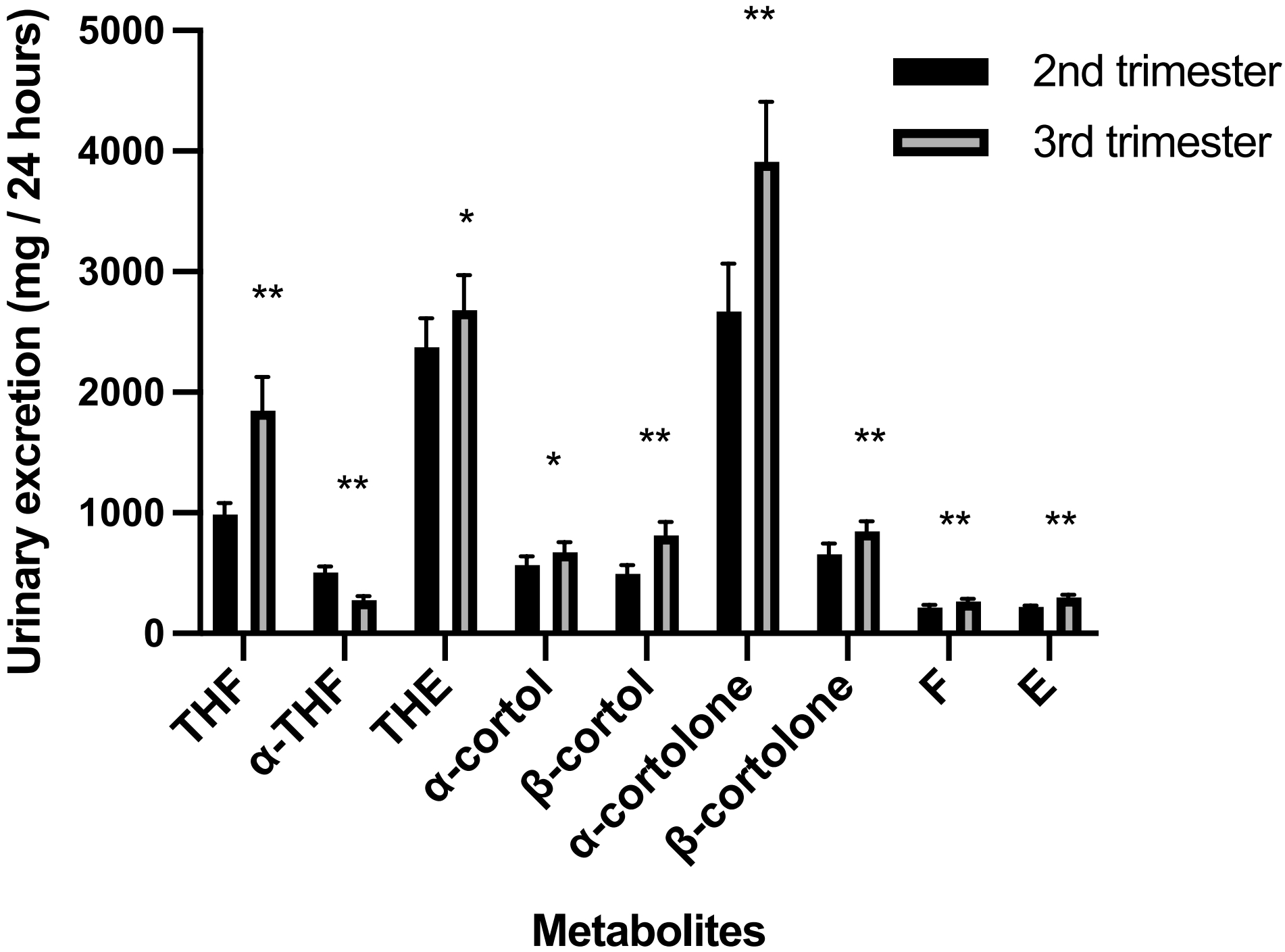
524 **glucocorticoid excretion or estimated enzymatic function**

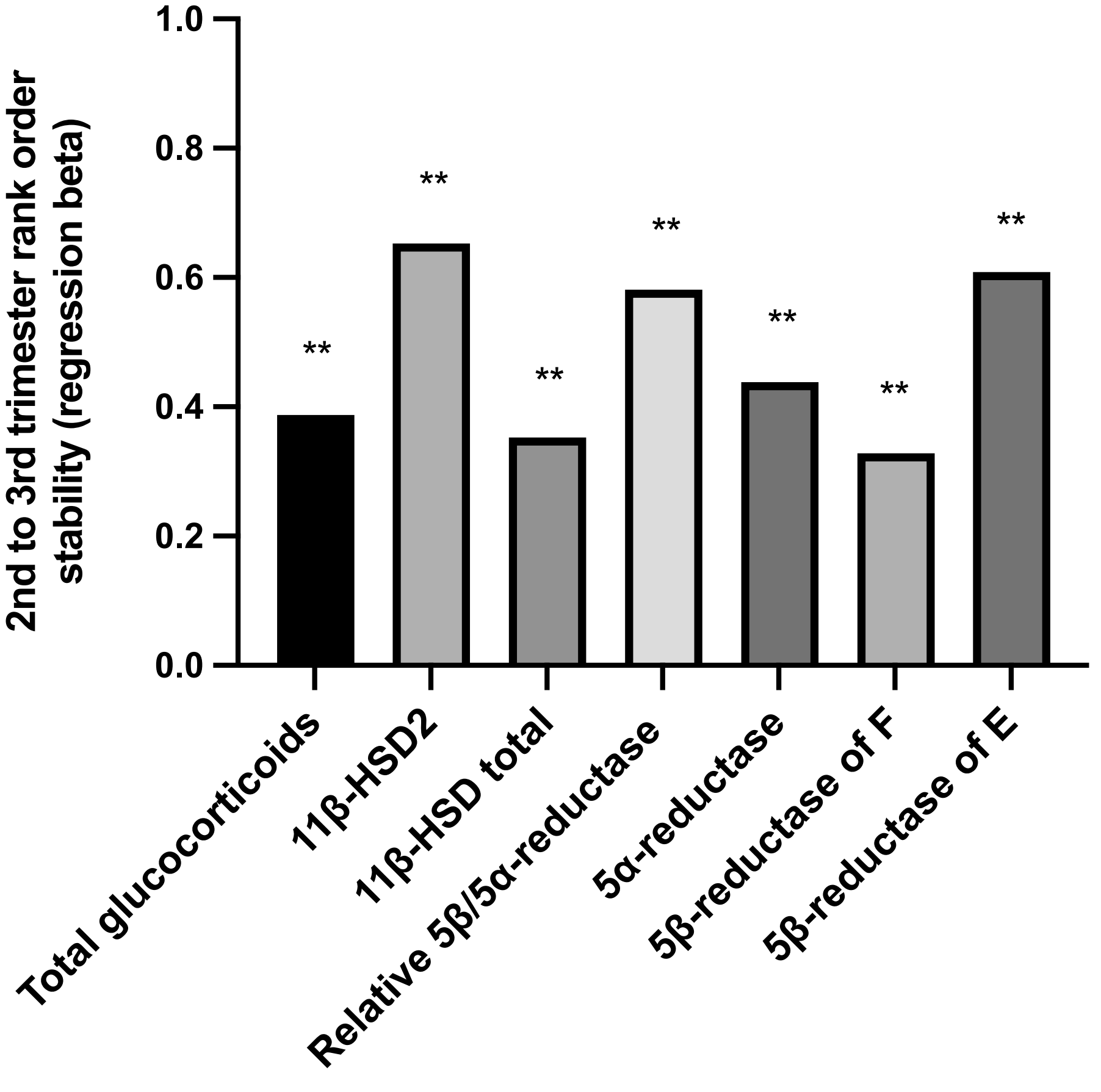
	Standardised Coefficient, β
Total urinary glucocorticoids	.387[2]
11β-HSD2 activity = F / E	.652[2]
11β-HSD total activity = (THF + α-THF) / THE	.352[2]
Relative 5β -reductase and 5α -reductase activity = THF / α-THF	.581[2]
5α -reductase activity = F / α-THF	.438[2]
5β-reductase metabolism of F = F / (THF + α-cortol + β-cortol)	.328[2]
5β-reductase metabolism of E = E / (THE + +α-cortolone+ β-cortolone)	.608[2]

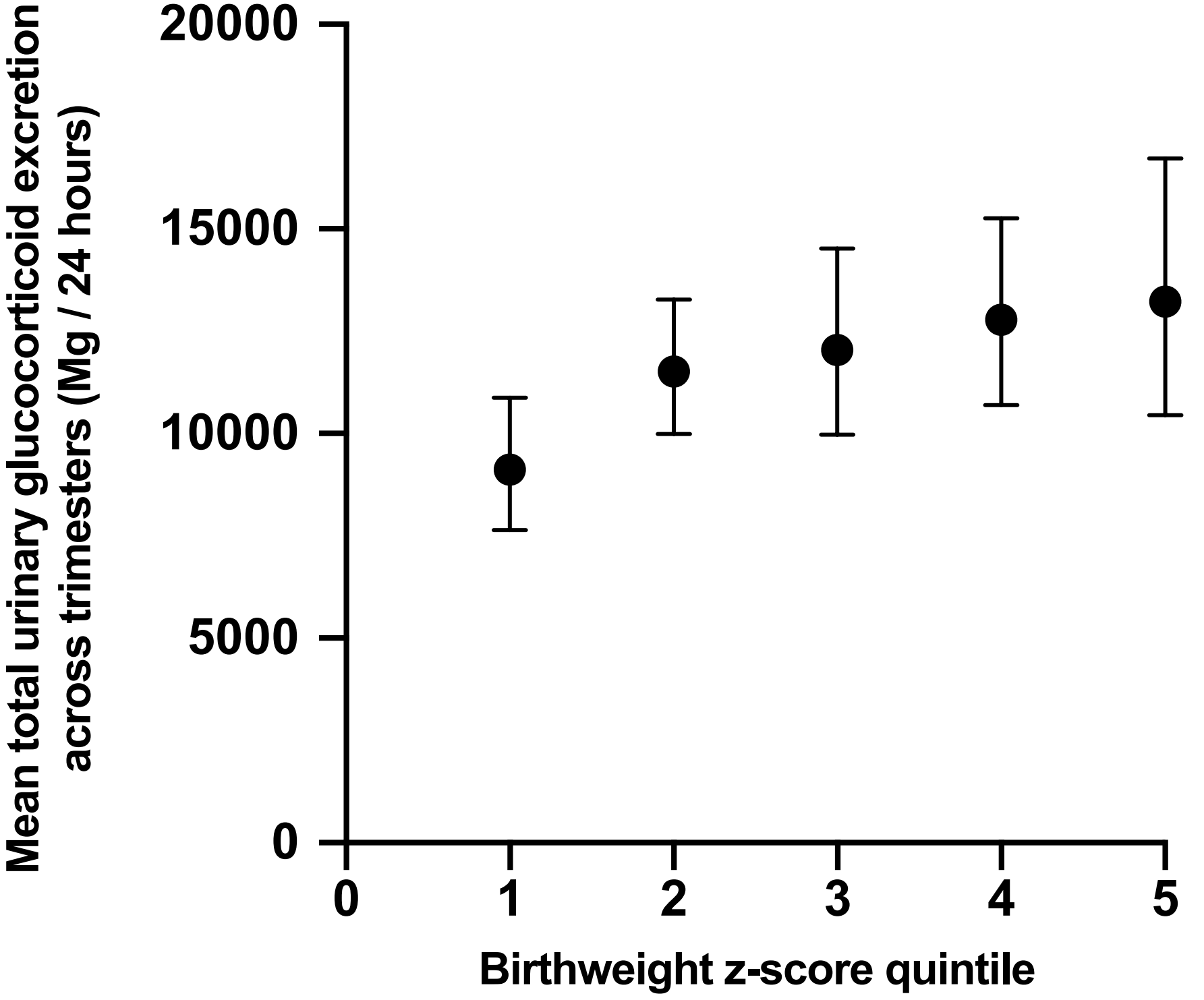
525

526 Adjusted according to the gestation of urine collection. [2] $p<0.001$


Cortisol
11β-HSD2
11β-HSD1
Cortisone
5β-reductase
3α-HSD
5α-reductase
3α-HSD
5β-reductase
3α-HSD
THF
α-THF
THE
20α-HSD
20β-HSD
20α-HSD
20β-HSD
α-cortol
β-cortol
α-cortolone
β-cortolone


Urinary excretion (mg / 24 hours)
5000
4000
3000
2000
1000
0
2nd trimester
3rd trimester
**
**
*
*
**
**
**
**
**
THF
α-THF
THE
α-cortol
β-cortol
α-cortolone
β-cortolone
F
E
Metabolites


2nd to 3rd trimester rank order stability (regression beta)
1.0
0.8
0.6
0.4
0.2
0.0
**
**
**
**
**
**
**
Total glucocorticoids
11β-HSD2
11β-HSD total
Relative 5β/5α-reductase
5α-reductase
5β-reductase of F
5β-reductase of E


Mean total urinary glucocorticoid excretion across trimesters (Mg / 24 hours)
20000
15000
10000
5000
0
0
1
2
3
4
5
Birthweight z-score quintile